Effect of water stress on root architecture in chile peppers (*C. annuum*) from contrasting origins

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CHAPTER 1: Local adaptation to drought of root phenotypes in chile pepper

Chile pepper (*Capsicum* spp.) has an undeniable importance to people around the world, especially those in chile pepper's native range—the Americas. Their cultural, nutritional, and economic value is critical for many communities. As climate change threatens to disrupt weather patterns in the regions dependent on this crop, it is even more important that we understand how chiles can withstand water deficits. For this, we turn to accessions native to areas with droughty climates. By studying the seeds and roots of these plants, we can observe how a plant that originates where water is scarce handles water deficit and how its response may differ from a plant of the same species that has evolved in an area where water is more readily available. With this knowledge, we may be better prepared to face the challenge of raising and breeding crops in a changing climate.

Chiles have long been culturally significant. Evidence suggests their use in the Americas could go back over 10,000 years (Bosland & Votava, 2012)—well before evidence of even maize usage (Ranere et. al., 2009). Remains found in caves in southern Mexico suggest that pre-Columbian people ate Capsicum fruits, both fresh and dried, as they camped while hunting and farming (Perry & Flannery, 2007). Slightly further south in Mexico, chile residue in pottery suggests that peppers were ground into a paste, which may have been used as a burial offering for the wealthy (Powis et al., 2013). In South America, Incans ate chiles with salt before drinking *chicha*, a fermented corn beer, in a tradition called *Llakhuay* (Guerrón, 2017). There are also ritual uses of chile that continue into the present. For example, some Yucatec Maya people celebrate the Assumption of the Virgin Mary by making pork in a sauce of blackened chiles, using earth oven techniques from over 3,500 years ago (Salazar et al. , 2012). Modern Yucatec

Maya also incorporate chile into a coming of age/healing ceremony, where chile fruits represent male genitals (Faust, 1998).

However, this flavorful fruit is not limited to ceremonial uses, as chiles are still incredibly important in modern Mexican cuisine and culture. From salsas to moles to candies, chile is a large part of what makes Mexican food Mexican (Katz, 2009). It has become part of both national and ethnic identities, as Mexican people tend to eat more chiles than the *gringos* in the United States can handle, and people in rural areas proudly do so to an even greater extent than the wealthier people of more urban areas in Mexico (Katz, 2009).

In addition to their cultural significance, chiles have great nutritional importance in some regions. Over 2 billion people today are still deficient in important vitamins and minerals such as iron, zinc, and vitamin A (Kantar et al., 2016; Olatunji & Afolayan, 2018). Those most affected by these deficiencies are women and young children, who often face anemia from iron deficiencies, mental retardation for want of iodine, or even blindness from a lack of vitamin A (Olatunji & Afolayan, 2018). This has led researchers to suggest that people in these regions add more chiles to their diet, since they are nutrient rich (Olatunji & Afolayan, 2018). Chiles contain carotenoids, flavonoids, phenolic acids, and vitamins A, C, and E, along with capsaicin, which has antioxidant properties (Rosa et al., 2002). Chiles also contain micronutrients like iron, zinc, and calcium, and may even aid in the absorption of these nutrients (Olatunji & Afolayan, 2018), making them an excellent option for people in areas with poor nutrition. In addition to these functions, chiles may also have antimicrobial, antiviral, and anticancer activities (Khana et al., 2014), qualities that make them valuable to all people, regardless of their nutritional status.

The economic value of chiles must not be overlooked either. As of 2009, over 1,350,000 tons of chiles were consumed in Mexico, with average yearly consumption reaching just under

30 pounds per capita (SIAP). Further north in the United States, almost 2 billion pounds of peppers were produced in 2016 (Minor, Bond, & Stewart, 2017). Even in Europe, chile imports have been increasing for the last few years, with significant increases expected in the next five years (Centre for the Promotion of Imports, 2019). The economic impact of this trade is substantial, with U.S. pepper production in 2018 valued at over $628 million (USDA NASS), and Mexican chile exports in the same year estimated to be worth over $1 billion (SAGARPA, 2017). In fact, chiles make up 3.5% of Mexico's agricultural GDP (SAGARPA, 2017).

With a significant impact on culture, nutrition, and the economy, chiles are clearly an important part of society, especially in the Americas. However, as climate change progresses, chile production in its native range may soon be in danger. Mexico has been identified as particularly vulnerable to the effects of climate change (United Nations Development Programme, 2021), with some areas predicted to lose up to 40% of their yearly precipitation and to have mean annual temperatures increase by as much as 4°C (Ministry of Environment and Natural Resources, 2016). This could spell certain death for crops that are not irrigated—a situation that is common in Mexico. A large portion of Mexico's total agriculture is rainfed during the spring and summer months (Conde, Ferrer, & Orozco, 2006), making them particularly vulnerable to drought. This crop loss could have a tremendous human cost as well, seeing as in 2011 about 13% of Mexican people were reliant on agriculture for their income (United Nations Development Programme, 2021). Chile production outside of Mexico is at risk as well, as major producers like India, China, Thailand, and Ethiopia (Agricultural Market Intelligence Centre, 2019) are all at risk of facing drought conditions by the end of the century (US EPA, 2017). With this much strain on their agricultural systems, it is almost certain that chile producers in these regions will suffer greatly at the hands of the hotter, drier conditions.

But of course, plants themselves will be the first to feel the effects of climate change. Water stress can be damaging at any life stage, but its effect can be especially great when it occurs as seedlings first attempt to emerge. Under low-water conditions, seed germination is slowed and reduced in many species, from grasses to legumes and trees (Muscolo et al., 2014; Toosi, Bin Bakar, & Azizi, 2014; Rahemi, Gholami, & Kholdebarin, 2010; Thabet et al., 2018; Okçu, 2005; Boureima et al., 2011; Khodarahmpour, 2011; Ahmadloo, Tabari, & Behtari, 2011; Shahriari & Davari, 2015). Some species also undergo an increase in seed lipid peroxidation under water stress, which leads to the degradation of the cell membrane (Li et al., 2013). Even after they develop into seedlings, plants are not free of the dangers of water stress. Young plants grown under a moisture deficit may have lower fresh (Okçu, 2005) and dry weights (Toosi et al., 2014), shorter hypocotyls (Toosi et al., 2014; Ranjan Kumar, Karjol, & Naik, 2011), decreased stomatal conductance and sap flow (Sato et al., 2003), limited photosynthetic activity (Fang-Lan Li, 2011), and fewer branches and leaves (Fang-Lan Li, 2011) when compared with seedlings grown in well-watered conditions. There is clearly a great toll taken when water is scarce.

However, there are some plants that may fare better under low water conditions due to their evolutionary history. As natural selection progresses, plants with traits that allow for more successful survival and reproduction under the prevailing conditions are selected for, and local adaptation occurs (Kawecki & Ebert, 2004). This means plants that evolved under certain conditions, like high altitude or drought, for example, will perform better in these environments than plants adapted to low-lying or well-irrigated environments (Mercer, Martinez-Vasquez, & Perales, 2008; Mercer & Perales, 2019). These locally adapted varieties, known as landraces, provide a source of genes that may offer pest and disease resistance, abiotic stress tolerance, or even unique nutritional qualities (Camacho Villa et al., 2006).

This diverse pool of genes is part of why landrace crops like the chiles in this study are so important. For production to remain viable under the new conditions created by climate change, plants will have to evolve or be changed through crop improvement. Such change will require genetic diversity for natural or artificial selection to act upon (Mercer & Perales, 2010)—something that is hard to come by in improved crops because of the genetic bottleneck created by domestication (Meyer & Purugganan, 2013). However, landraces have not undergone as strong selection as formally bred varieties have, thereby maintaining genetic variation that may equip them to grow in the conditions created by climate change (Mercer & Perales, 2010). For example, it has been shown that chiles native to drier environments delay germination under drought stress longer than those native to areas with more available water (Bernau et al., 2019). This delay of germination may seem detrimental to the crop, but it serves as a drought avoidance mechanism that allows plants to postpone growth until conditions are more favorable (Clauss & Venable, 2000). This is just one example of how evolution has led to the local adaptation that allows chiles to remain fit in harsh environments.

Aside from these evolutionary reasons, plants are also slow to germinate under soil water deficits because of pure physiological constraints. Seeds must imbibe water to extend the radicle and synthesize enzymes that allow the endosperm to be utilized (Ahmadloo et al., 2011), so the lower metabolic activity and turgor pressure that result from drought conditions will prevent premature germination. However, some seeds of desert plants are able to modify their chemical compositions to better withstand droughts, like the Chinese native *Eremosparton songoricum* (Li et al., 2013). As the level of water stress increases, seeds of this xerophyte develop higher levels of catalase, which is thought to serve as an antioxidant that helps prevent oxidation damage to the seed (Li et al., 2013).

As seedlings, plants develop other mechanisms to survive water-limited conditions. One strategy is to reduce stomatal conductance to curb water loss through transpiration (Sato et al., 2003). Another is to develop a higher stem density to reduce the risk of xylem cavitation (Markesteijn & Poorter, 2009). There also tends to be a reduction of seedling shoot growth under drought conditions, but an increase in the amount of dry mass allocated to the roots (Ranjan Kumar et al., 2011; Fang-Lan Li, 2011), causing in increase in the root-to-shoot ratio. This is indicative of the importance of roots in a plant's survival, especially when so much depends on the ability of plants to seek out water within the soil.

However, simply having a large mass of roots does not guarantee success in a drought. A key factor in a plant's survival is the spatial configuration of its roots in the soil, known as its root architecture (Wasaya et al., 2018). Root architecture is closely linked to a plant's water uptake ability (Hernández et al., 2010), meaning that design could actually be a matter of survival when the soil's water potential is low. As may be expected, plants with deeper rather than shallow roots have better rates of survival (Fensham & Fairfax, 2007) and higher yields (Carretero, Bert, & Podestá, 2014) under drought conditions. However, this is not simply a case of a "more is more" philosophy. For example, *Arabidopsis* plants that are more adept at handling water stress actually have fewer lateral roots (Riedelsberger et al., 2016), and more drought-tolerant maize plants invest less in horizontal expansion (Lynch, 2018) than their less tolerant counterparts. Instead, they tend to allocate more resources towards longer, more streamlined roots that allow them to access water stored deep in the soil (Lynch, 2018).

Chiles specifically might not be expected to behave any differently than other plant species under water deficit conditions, but there has been very little research done on their root systems. It has been shown that their root-to-shoot ratio significantly increased as a result of

drought stress (Phimchan et al., 2012), indicating that there is more biomass allocation directed towards underground growth and water acquisition. However, it has not yet been explored whether this greater mass is found in an expanse of thin, widespread fibers or in a few large vessels driven deep into the soil. Also unexplored is whether these changes occur once water is limited or how these effects may differ between accessions that have evolved under different conditions. In this study, we hope to answer these questions and shed light on the hidden half of chile plants.

To do so, our objective is to measure the effect of soil water deficit on germination and root architecture of chile seeds from divergent origins. In particular, we want to know if all accessions of chile pepper show similar germination and root architecture characteristics and if these traits shift in the same manner with water deficit for all accessions. We anticipate that the seeds of the chile native to the drier region will not germinate as quickly as those of the chile from the area with more available water, especially under low-water conditions, and that there will be differences in the root architectures of our chile accessions could become more prominent as water availability declines. We expect this to take place in the form of root systems that devote fewer resources to lateral roots and more to long axial roots that can reach water deep in the soil.

CHAPTER 2: Effect of water stress on root architecture in chile peppers (*C. annuum*) from contrasting origins

Abstract

Chile peppers (*Capsicum* spp.) have significant cultural, economic, and culinary impact throughout much of the world. However, chile pepper production is vulnerable to climate change and the accelerated risk of drought. To combat these setbacks in productivity, growers will need to utilize chile accessions that are adapted to better handle water stress. These more drought-hardy chiles may be found in Mexico, a center of domestication for chile pepper, which has many local varieties (landraces) that possess adaptations to the environments where they evolved. The accessions that have evolved in arid climates may possess adaptations to droughty conditions that allow them to more efficiently mine for water, as will be necessary in the hotter climate we will soon be facing. One metric strongly tied to water uptake ability is a plant's root architecture (the spatial configuration of roots within the soil), but it has not been studied extensively, especially in broadleaf crops like chiles. To explore the adaptations of chile root systems to the level of moisture in their environments of origin, landrace germplasm was selected from areas around Mexico with high, moderate, and low precipitation levels. Plants were germinated under uniform conditions, then grown under two levels of water stress and harvested five weeks after planting. Their roots were then scanned and analyzed to identify differences in root architecture between genotypes, between water stress levels, and among genotypes in response to water stress. We predicted that the accessions with higher water uptake ability would have lower root to shoot ratios, higher specific root lengths, and lower surface areas than the less drought-tolerant accessions. We also expected that water stress would enhance traits associated with high water uptake. We found that chiles originating from environments of moderate precipitation tended to be the tallest, and that plants grown under droughty conditions

had shorter, lighter shoots, and roots that were thinner, lighter, and had lower total lengths than plants in the control group. We also determined that specific root length is dependent upon the interaction between a chile's genetic makeup and its environment, although no clear trend is apparent vis-à-vis its environment of origin. Further research will be necessary to clarify our results and to increase the understanding of beneficial root architecture traits that may someday be bred into commercial cultivars.

Introduction

Chile peppers, a culinary staple for many of us, play many important roles for people around the world. Many cultures have adopted the chile as a way to add color and spice to their cuisines, but it is especially important in giving Mexican food its signature appeal (Katz, 2009). This gives the chile important cultural and ritual meanings for many Mexican people today (Katz, 2009; Faust, 1998; Salazar, et al., 2012), as it has in the past (Guerrón, 2017; Powis et al., 2013). It is also rich in nutrients such as vitamins A, C, and E, iron, zinc, carotenoids, flavonoids, antioxidants, and phenolic acids (Rosa et al., 2002; Olatunji & Afolayan, 2018), making it an ideal food source for malnourished populations (Olatunji & Afolayan, 2018).

However, the changing climate is putting all this at risk. Mexico has been designated an area of particular vulnerability to the effects of climate change (UNDP, 2021). Some areas may warm by as much as 4°C while losing up to 40% of their yearly precipitation by the year 2075 (Ministry of Environment and Natural Resources, 2016). Other major producers of chile, like India, China, Thailand, and Ethiopia (Agricultural Market Intelligence Centre, 2019) may also experience more droughty conditions in the near future (US EPA, 2017). Given the detrimental effect water stress tends to have on yield and quality, this could cause major disruptions to chile supplies worldwide.

However, some chiles may not fare so poorly under such arid conditions. Certain landraces, or locally adapted varieties, may carry genes that allow them to better withstand abiotic stresses like drought and high temperatures (Camacho Villa et al., 2006). This would have come about as a result of natural selection acting on the diverse gene pool available in Mexico, a center of domestication for chiles. As evolution progressed, it selected for chiles with traits that were advantageous under the local conditions (Kawecki & Ebert, 2004). This should lead those chiles that evolved in drier climates to perform better in these types of environments than landraces adapted to climates with more available moisture (Mercer et al., 2008; Mercer & Perales, 2019).

One of the traits that has likely been selected for is root architecture, or the spatial arrangement of roots in the soil. Root architecture plays an integral role in a plant's water uptake ability (Hernández et al., 2010), and therefore its survival (Wasaya et al., 2018). Growing deeper, longer roots that penetrate to reach water stored in the soil can help plants survive and reproduce under droughty conditions, and even the angle at which its roots grow can be important in determining drought tolerance (Riedelsberger et al., 2016). However, it is not enough for a plant to simply have more or longer roots; it must judiciously allocate its resources to maximize water uptake while conserving energy (Lynch, 2018). The model that appears to be most efficient for this purpose, at least in monocot crops, is composed of long, streamlined roots that access water deep in the soil, rather than a system of fine, fibrous lateral roots (Lynch, 2018) that are typically desirable in non-drought conditions because of the additional surface area they provide for water uptake (Smith & de Smet, 2012).

The plant's ability to tailor its root architecture to be best suited for its environment requires a degree of phenotypic plasticity, which produces a trait whose expression is dependent upon

both the plant's environment and its genome (West-Eberhard, 2008). Our expectation is that an adaptive plastic response to drought in chiles would include a reduction in fine lateral roots in comparison to chiles grown in less arid environments where such roots are advantageous for more plentiful water uptake. However, we do not yet know how much of a plant's root architecture is determined by genetic makeup, environment, or the interaction of the two factors; so, we still know little about how a plant's environment of origin determines its root architecture in response to drought stress.

This question of genetic versus environmental determination of root architecture is what drives our investigation. In this study, we examined the root architectures of multiple landrace accessions of chiles from environments of high (829-935 mm), moderate (621 mm), and low (530-575 mm) precipitation under control and simulated drought conditions. Our aim was to discover whether accessions from all environments respond similarly to water deficit or if there might be differences in their responses due to their presumably distinct evolutionary histories. We expected that plants grown under droughty conditions would have longer, thicker, heavier roots than their well-watered counterparts—traits that are associated with higher water uptake. We also anticipated that shoots of plants grown under water deficit would be shorter and have lower biomasses than plants that did not undergo water stress. Our prediction was that there would be differences in root architecture with regard to accession as well. Finally, chiles native to more arid environments were expected to exhibit greater change in root morphology in response to drought stress than those from areas with more precipitation.

Methods

Germplasm

The ten chosen accessions represent germplasm from a broad range of environments and production systems; a full list of accessions can be found in Table 1. The range of moisture for all areas sampled was 154-1690 mm of precipitation, so the regions where the accessions in this study were found cover about the middle 26% of the gradient. Seed was originally collected from across Mexico between 2013 and 2019. The selected accessions originate from environments along a gradient of precipitation and are divided into categories of high, low, and moderate moisture as determined by the precipitation of their wettest quarter. We chose this metric because it typically coincides with the season of active growth for chiles, meaning it would have the greatest impact on their development. To avoid confounding environment of origin with production system, we chose accessions primarily obtained from backyards. Additionally, we included one accession from a forest and one from a commercial greenhouse production system; the latter accession is considered to be in an environmental category of its own, as it is not subjected to the ambient environmental conditions of the collection site. Previous research (P. Leon, unpublished) also suggested that the greenhouse accession had a unique root structure, making it of interest for comparison with the other accessions. We grew original seeds of all accessions under uniform conditions in a greenhouse in the United States prior to this experiment to increase seed and eliminate maternal effects in the resultant seeds; we then used these seeds for this experiment.

Germination

We plated seeds on October 6th, 2020. To surface disinfest the seeds before incubation, we soaked germplasm from all accessions in a 0.825% sodium hypochlorite solution for 10 minutes, then rinsed it with sterile water. Then, using aseptic technique whenever possible, we

placed the seeds in petri dishes filled with about 75 grams of autoclaved Quikrete Premium play sand (The QUIKRETE Companies, Atlanta, GA, USA) and 15 mL of sterile water, and we sealed the petri dishes with Parafilm (Bemis Company, Inc.., Neenah, WI, USA). We calculated the approximate number of seeds of each accession that would ensure sufficient germination for the experiment using data from a preliminary viability test and placed a maximum of 25 seeds per plate to ensure adequate moisture. The plates were sealed and then arranged randomly on a tray and placed it in a Conviron G30 germination chamber (Conviron, Winnipeg, Manitoba, Canada) kept at 30°C during the day and 20°C at night with 12 hours of light provided between noon and midnight each day. We checked all plates frequently to monitor germination.

Planting

Once about four seeds of each accession had germinated, we planted the first block on October 16th, 2020, and the second and third blocks on October 19th, 2020. We planted germinated seeds with only radicles emerged at a depth of about 0.25 cm with their radicles pointing down; we planted the seeds with more developed shoots, which accounted for about 15% of germinated seedlings, such that the soil reached about midway between the transition of root to shoot matter. We planted all seeds in a mixture of 30% play sand and 70% potting soil (by volume) that we thoroughly mixed in a small cement mixer. We chose this mixture because of its ability to retain an appropriate amount of moisture while allowing for easier cleaning of harvested roots. We then filled black plastic six-liter pots that had drainage holes in the bottom with media. We thoroughly moistened media before planting and gently watered the seeds once they were in place.

Experimental Setup

This experiment took place in the Howlett Greenhouse at The Ohio State University in Columbus, OH. During the day, we kept the greenhouse between 22.8-23.9°C, and at night, we kept it between 20.0-23.9°C. We provided supplemental lighting using overhead PAR Source 1000-watt metal halide lamps from 11.5 hours before dusk to a half hour before dusk; this way, 11 hours of supplemental light was provided, in addition to natural light, with one hour of natural light only occurring from a half hour before dusk to one half hour after dusk. Daylengths began to shorten noticeably in late October. We arranged replicates into three blocks as depicted in Figure 1. Pots were arranged in the same configuration on an unlit bench for the first ten days of the experiment, at which point we moved them to the bench shown in Figure 2 to be positioned more directly under the overhead lights and further from the outside wall.

In this randomized complete block design, there were three blocks within which we randomly assigned the combinations of pepper accession, root harvest date, and water availability. Our main focus was to determine the effects of water deficit on the root architectures of diverse accessions of peppers. The effects of harvest date were a secondary focus within this goal, so only accessions Ca0057 and Ca0181 had both early and late harvest treatments; we harvested all other accessions at the later date. The early harvest took place three weeks after planting, and the late harvest occurred about five weeks after planting.

Irrigation

There were two levels of water availability in this experiment, one representing a well-watered control and the other representing water deficit. The pots in the control group were kept within 1 standard deviation of 70% of field capacity, and pots in the water deficit group were maintained within 1 standard deviation of 30% field capacity. We determined field capacity and

its standard deviation prior to the experiment using five randomly chosen pots filled with media and calculating the average mass of water they held four hours after being watered to saturation. For the first four days after planting, we kept all pots well-watered by hand to allow for seedling establishment. After this period, we applied the control and water deficit treatments. To maintain the pots within the range of weights, we placed each pot on a scale every day and weighed it. We watered pots that were more than one standard deviation outside the appropriate range by hand using a small beaker filled with tap water from the greenhouse facility until their weight was once again within range. From November 13th to November 15th, we could not collect data because there were issues with the accuracy of the scale. There were some irregularities (pots that saturated too soon, others that never dried out enough); we noted each of these instances. We did analyze pot weights at the end of the experiment, though, and the treatments were significantly different (control: 4.07 ± 0.0426 kg; treatment: 3.34 ± 0.0426 kg). We recorded emergence date for each plant as well.

Early Harvest

The only treatments we harvested during the early collection were accessions Ca0057 and Ca0181, both control and water deficit treatments. We harvested plants from Block 1 on November 6th, 2020 and we harvested Blocks 2 and 3 on November 9th, 2020. Before the seedlings were cut, we measured the heights of all plants in the block from the soil's surface to the node of the highest petiole. Then, we weighed and watered all pots as usual. After about an hour and a half, we weighed the accessions being collected again just before their shoots were cut at the soil level. We recorded the weights of the shoots and placed them in paper bags in a dryer at about 55°C until they were fully dry, at which point we recorded their dry weights.

Immediately after the shoots were cut, we removed the roots by sticking our hands directly into the pots and scooping out the central portion. Then, we gently loosened the debris from the roots by hand; we also collected and cleaned all root segments that had broken off in the soil. Once roots were mostly free of soil, we further cleaned them in tubs of tap water, then placed them in plastic zippered bags and weighed them before storing them in a refrigerator until they could be scanned.

Late Harvest

The second harvest took place between November 20th, 2020 and November 25th, 2020, by order of block number. Because of the extended period of time it required, plants that were not harvested were weighed and watered as usual to maintain proper experimental conditions. This harvest included all remaining treatments. Once again, we measured shoot heights and pot weights before shoots were harvested and weighed. We then stored shoots in paper bags in a dryer at about 55°C until they were completely dry; then, we recorded their dry weights.

Immediately after its shoot was harvested, we also exhumed the roots of each plant. Because the root systems were much longer at this point than at the first harvest date, simply scooping the roots up out of the soil was no longer feasible. We ultimately determined that cutting the pot in half and removing it from the outside of the soil mass was the best approach. Once the pot was removed, we gently removed the media by hand, working from the outside of the soil mass in towards the center. We also collected roots that had broken off from the rest of the root system. We then further cleaned all roots in tubs of water as with the first harvest, stored them in plastic zippered bags, weighed them, and stored them in a refrigerator until they could be scanned.

Scanning & Drying

We scanned roots from the first block between December 4th, 2020, and December 7th, 2020. We scanned roots from the second block on December 7th, 2020, and we scanned roots from the third block on December 8th, 2020. To obtain images of the roots, we floated the cleaned roots in shallow tubs of water so they could be gently spread out. We then positioned a clear plastic clipboard underneath the roots in the tub, and once the roots were thoroughly spread, we slowly lifted the clipboard up out of the water with the roots held to it. We placed all stray roots on the clipboard and gently patted both sides completely dry. We then placed the clipboard directly on the glass of the scanner (roots side up) with a piece of black cardboard on top of the clipboard in the scanner to provide a contrasting backdrop. We scanned the roots using an HP Scanjet 4850 flatbed scanner (The Hewlett-Packard Company, Palo Alto, California, USA) and WinRHIZO Pro software (version 2007d) (Regent Instruments Inc., Canada). After we scanned all roots in each block, we placed them in paper bags and put in them the dryer at about 55°C and then weighed them to find their dry weights.

Analysis

We carried out all analyses in RStudio version 3.6.1 (RStudio, PBC, Massachusetts, USA) using the lmer package. We created two models; in the first, block was a random factor, and accession, irrigation treatment, and their interaction were fixed factors. The second included block as a random factor as well, and had environment of origin, irrigation treatment, and their interaction as fixed factors. We also added accession within environment and the interaction of irrigation treatment and accession within environment as random factors in this latter model. We first ensured the data met the assumptions necessary for analysis of variance by checking the residuals of each model. Two variables, shoot dry weight and root dry weight, required a log transformation due to a lack of normality in their residuals in the first model. Shoot dry weight

data required a square root transformation in the second model. We then performed analyses of variance to determine significant main effects. Once these were found, we further examined them by performing means separations.

Results

Effects of environment of origin

Analysis revealed that a chile's environment of origin did significantly impact its shoot height at both three and five weeks after planting (Table 2). At three weeks, plants from areas with moderate precipitation were about 2 cm taller than plants native to regions with high precipitation, and about 1.5 cm taller than those from regions with low precipitation (Figure 3a). At five weeks, chiles from the moderately rainy environment were still significantly taller than those native to the areas with high precipitation (Figure 3b). The chiles from the driest climate were no longer significantly taller or shorter than either other group. Environment of origin did not have a significant effect on shoot dry weight, root dry weight, root length, emergence date, root diameter, specific root length, or root to shoot ratio.

Effect of irrigation and its interaction with environment of origin

Irrigation did not have a significant effect on plant height at three weeks, but it did have a significant effect on the heights of the chiles at five weeks (Table 2). Those grown under the simulated drought conditions were significantly shorter than those in the more well-watered control group, with the former being about 1.6 cm shorter than the latter. (Figure 4a). Shoot dry weight was significantly affected by drought treatment as well (Table 2), with plants in the control treatment weighing about 0.1 g more than those in the water deficit treatment (Figure 4b). In addition, the factor of water availability significantly impacted root length (Table 2).

Chiles grown with less water had shorter total root lengths than those in the control group (Figure 4c). Treatment did not have a significant effect on root dry weight, emergence date, root diameter, specific root length, or root to shoot ratio. However, there were trends toward higher root dry weights and larger root diameters within the control treatment (Figures 4d,e). No significant interaction between environment of origin and treatment was detected for any variable (Table 2).

Effects of accession and its interaction with irrigation

When we ran a model with accession as a fixed factor, we found that accession had a significant impact on certain traits. At five weeks after planting, accession Ca0456, which is native to an environment with moderate precipitation, tended to be 5-6 cm taller than accessions Ca0446 and Ca0435, which come from environments of high precipitation (Figure 5a). Specific root length (SRL) was also affected by accession, with Ca0435 having significantly higher values than Ca0181 (an accession from a dry environment) (Figure 5b). There was also a significant interaction between treatment and accession for specific root length (Figure 6a). No clear trends can be determined, but it is apparent that not all accessions responded in the same way to drought stress. Some exhibit higher specific root lengths under water stress; others have higher specific root lengths under control conditions; still others have almost no difference in specific root length between treatments. We checked for possible interactions occurring with all other variables as well, but none were significant (Figures 6b-j).

Discussion

Some results of this experiment were in line with our expectations. Emergence date, for example, was not anticipated to vary among accession, treatment, or environment of origin, as we selected germinated seeds in advance so all accessions and treatments would be at the same developmental stage at the time of harvest. We also saw that plants grown under simulated drought had shorter shoots with less biomass. Other results, however, were more unexpected. The environment of origin of the chiles only had any real effect on their heights, with plants from environments of moderate precipitation tending to be the tallest. We also found that drought did not impact plant height until five weeks after planting but was correlated with shorter total root lengths. Some of the most surprising results of this study were the trends of chiles grown under drought having lower root dry weights and thinner root diameters, as these directly contradicted our expectations. Our predictions were also incorrect about root to shoot ratios and specific root lengths, which were not significantly affected by environment of origin, treatment, or the interaction between the two factors. However, we did see a significant interaction between treatment and accession with regard to specific root length. This data does not give us a clear picture of how the root architectures of chiles of different origins generally change in response to drought stress, but it does highlight multiple interesting opportunities for continued research in the future.

The reduction in shoot growth that we observed under simulated drought was unsurprising, as the abscisic acid produced under drought stress is known to inhibit shoot growth (Akhtar & Nazir, 2013). However, it was interesting that drought did not impact plant height until five weeks after planting, which may suggest some delay in the plant's response to stress. Some pots took more time to dry down to their treatment weight after being well-watered for

seedling establishment, so this may have delayed the onset of the chiles' stress response as well. Height was also significantly impacted by environment of origin, with the greatest heights reached by plants native to environments of moderate precipitation. This could indicate that these plants were better able to acclimate to both well-watered and droughty environments and thereby invest more resources into shoot growth.

The shorter total root lengths of plants grown under drought stress are more difficult to interpret. Because we do not know how the total length was distributed, we cannot definitively say whether the decreased length manifests in a reduction of lateral roots as we expected, or as roots with the same amount of branching that simply do not reach as deep into the soil. Should the reduced length be due to roots not reaching as deep into the soil, this may be a response to the containers in which the chiles were grown. There is some evidence that while deeper-reaching roots improve water capture in a field setting, they may not have the same utility in small containers such as those used in this experiment (Lynch, 2018). The uncertainty of how root length is distributed also complicates our interpretation of specific root length. Future studies could include a measure such as maximum length of longest roots to better elucidate where the reduction in length occurs.

The trends of chiles grown under drought having lower root dry weights and thinner root diameters, while not considered significant in this work, are certainly worth considering. Many other studies on roots do use an alpha value of 0.1 because of the high variability in root data, so we will evaluate these trends seriously. While the thinner root diameters are difficult to explain, the lower root biomasses of the chiles grown under drought may be a result of the plant only producing a few roots that reach deep into the soil rather than a mass of shallower roots; this is in line with our expectations. The traits of root dry weight and root diameter could be interesting to

research further in future studies, especially if different methods become available for root analysis that improve the accuracy of root length and diameter measurements.

Root to shoot ratios and specific root lengths were not greatly impacted by treatment, environment of origin, or the interaction between the two, which was surprising because we expected that these would be especially responsive and indicative of local adaptation. This means these traits are neither determined by genetic makeup nor their environments, and that there is no real adaptive plasticity based on evolutionary origin as we had anticipated. However, there is still the issue of specific root length not taking into account the distribution of the root length, so this may not be an entirely accurate assessment.

The interaction that appeared between treatment and accession with regard to specific root length was interesting in that there were clear differences in the way many of the accessions responded to water deficit, but there was no clear trend among them. Most of the variation is likely due to the highly plastic response of accession Ca0435, which is native to the environment with the greatest amount of precipitation. Other accessions from regions with relatively high precipitation did not respond similarly, however, so it appears that each accession may allocate its resources differently when experiencing drought stress. With this in mind, it is difficult to determine what the general response of root architecture in chiles is to drought.

Ca0435 is especially interesting not only because it demonstrates strong plasticity, but also because it had the lowest height at five weeks after planting, and the highest specific root length. This suggests that this accession characteristically has relatively short shoots with roots that have less length per mass (but similar biomass to other accessions) under droughty conditions and add more length per mass under well-watered conditions. If the distribution of

this length could be determined in a future study, this accession may serve as a valuable model of adaptive plasticity.

Given that so many of our expectations for this study were not met, it is important to consider the factors that could have led the roots to develop differently than anticipated. Firstly, there could be variables in their evolutionary histories that we did not previously account for, such as variation between the soil fertility and texture of their native regions, backyard environments and the amount of human care they may have received, and the importance of defense compounds. These factors may exert different selective pressures that take precedence over the chile's response to water stress; for example, a plant may devote more resources towards shoot growth even in an arid climate if it evolved to become quickly recognizable by humans so they would be encouraged to irrigate it. We must also remember that this study examines chiles only in their early life stages. Mechanisms of drought tolerance may vary between developmental stages, with drought tolerance at one stage not necessarily indicating the same tolerance during other stages (Bernau et al., 2020). This could mean that while some of the young chiles in this study may not have exhibited traits linked to drought tolerance at this stage, they may develop such adaptive phenotypes late in their development.

We also acknowledge that our sample size of only three accessions from each environment is somewhat small, and these environments, though differentiated amongst themselves, all have relatively moderate levels of precipitation when compared to all environments across Mexico. Further studies would do well to include an even wider range of accessions in their analyses. There is also the possibility that our drought treatment was not sufficiently intense, thereby reducing any differences that might appear under more severe stress. A greater number of replications would also be helpful in future studies as well because of the

large amount of variability in root data. Finally, most studies that our hypotheses were based upon examined only monocot crops, so it is possible that chiles, being dicots, have different root architecture responses to water deficit. Additional studies will be necessary to clarify and expand upon the research presented in this volume.

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Tables

Table 1: Accessions of *C. annuum* used in this experiment and their origins

Accession	Location	System	Type	Name	Annual Precip (mm)	Precip Seasonality	Precip of Wettest Quarter (mm)	Precip Class
Ca0057	Oaxaca Central Valleys	Commercial greenhouse	Landrace	De Agua	666	89.7	349	GH
Ca0181	Yucatan	Backyard	Landrace	Paradito	1149	68.0	544	Low
Ca0256	Oaxaca Coast	Backyard	Landrace	Chilgole	999	110.4	575	Low
Ca0303	Oaxaca Coast	Forest	Wild	Chile de Monte	892	115.0	530	Low
Ca0435	Chiapas Coast	Backyard	Landrace	Miraparriba	1534	110.3	935	High
Ca0436	Chiapas Coast	Backyard	Wild	Tipinchile	1345	111.6	829	High
Ca0446	Chiapas Coast	Backyard	Landrace	Miraparriba	1345	111.6	829	High
Ca0454	Oaxaca Canadas	Backyard	Landrace	Achilito de Monte	1142	87.3	621	Mid
Ca0456	Oaxaca Canadas	Backyard	Landrace	Huacle Rojo	1142	87.3	621	Mid
Ca0457	Oaxaca Canadas	Backyard	Landrace	Huacle Amarillo	1142	87.3	621	Mid

Table 2: ANOVAs (F value$_{\text{numerator df, denominator df}}$) of various traits for chile pepper (*C. annuum*) landraces grown under well-watered and water deficit conditions in Howlett Greenhouse at The Ohio State University in 2020.

Source	3-week height	5-week height	Shoot dry weight†	Root dry weight	Emergence	Root length	Root diameter	SRL	Root to shoot ratio
Environment of origin	$10.95_{2, 48}$***	$6.44_{2, 5.9}$*	$1.13_{2, 4.9}$	$1.22_{2, 43.8}$	$0.21_{2, 5.9}$	$0.61_{2, 44.2}$	$0.30_{2, 5.7}$	$2.29_{2, 6.0}$	$0.42_{2, 6.2}$
Treatment	$2.64_{1, 48}$	$5.50*_{1, 42.6}$	$5.06_{1, 35.7}$*	$2.92_{1, 43.4}$+	$0.35_{1, 38.9}$	$4.41_{1, 43.9}$*	$3.37_{1, 39.4}$ +	$1.81_{1, 39.5}$	$1.72_{1, 42.7}$
Environment x Treatment	$0.44_{2, 48}$	$0.26_{2, 42.6}$	$0.29_{2, 36.9}$	$1.26_{2, 44.7}$	$0.09_{2, 39.9}$	$0.84_{2, 44.7}$	$1.92_{2, 39.8}$	$0.46_{2, 40.0}$	$2.36_{2, 42.7}$

+ P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001

† data transformed to its square root

Table 3: ANOVAs (F val$_{\text{numerator df, denominator df}}$) of various traits for chile pepper (*C.annuum*) landraces grown under well-watered and water deficit conditions in Howlett Greenhouse at The Ohio State University, 2020.

Source	3-week height	5-week height	Shoot dry weight$^{\Delta}$	Root dry weight$^{\Delta}$	Emergence	Root length	Root diameter	SRL	Root to shoot ratio
Accession	$3.30_{8,36}$**	$3.47_{8,36}$**	$1.25_{8,32.6}$	$1.18_{8,36}$	$1.98_{8,32.3}$+	$0.86_{8,32.5}$	$1.27_{8,32.6}$	$3.45_{8,33.1}$	$1.43_{8,36}$
Treatment	$1.83_{1,36}$	$4.72_{1,36}$	$3.59_{1,32.2}$+	$2.02_{1,36}$	$0.34_{1,31.8}$	$3.44_{1,32.1}$+	$2.56_{1,32.4}$	$3.69_{1,32.8}$+	$1.50_{1,36}$
Accession x Treatment	$0.49_{8,36}$	$0.80_{8,36}$	$1.36_{8,32.7}$	$1.40_{8,36}$	$0.37_{8,32.4}$	$0.72_{8,32.5}$	$2.42_{8,32.7}$	$2.48_{8,33.1}$	$0.66_{8,36}$

+ P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001

Δ data transformed to its log

Figures

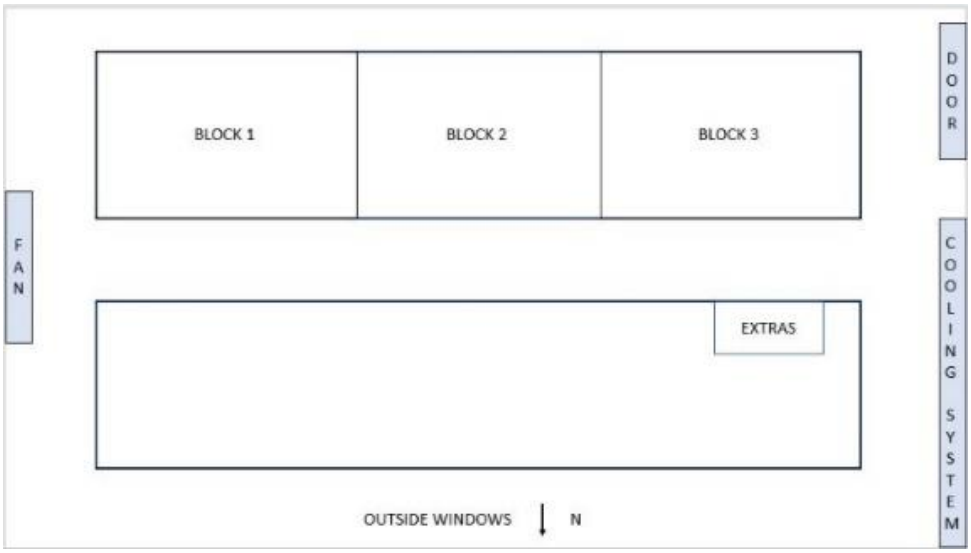


Figure 1: Setup of experimental blocks in Howlett Greenhouse, The Ohio State University, 2020.

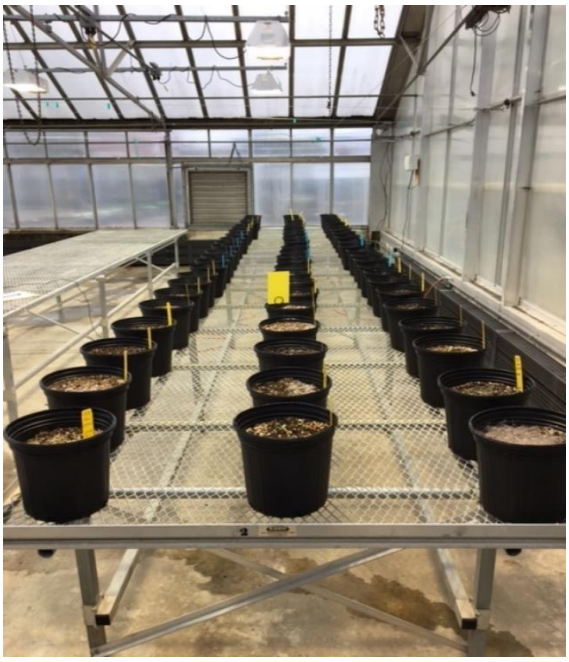
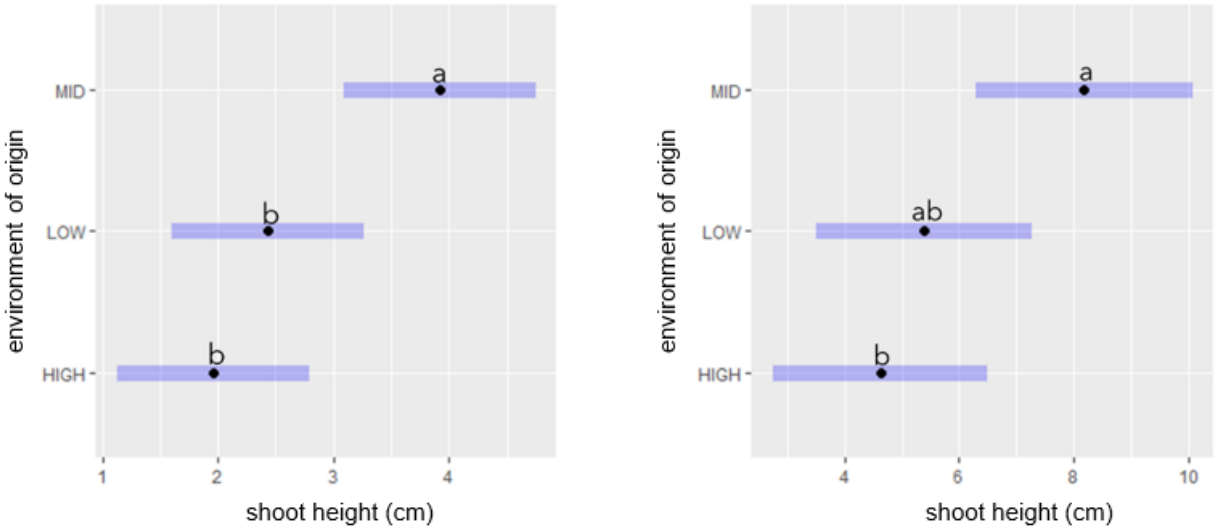


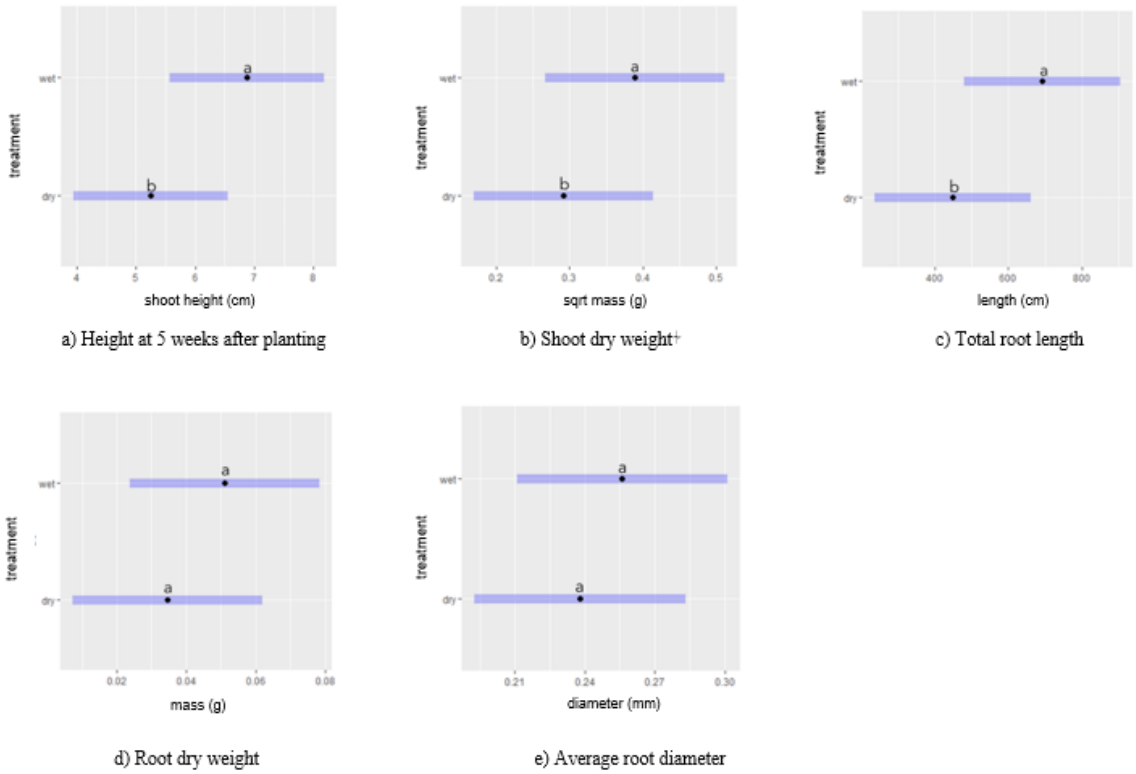
Figure 2: Arrangement of pots on greenhouse bench in Howlett Greenhouse, The Ohio State University, 2020.



a) Height at 3 weeks after planting

b) Height at 5 weeks after planting

Figure 3: Least squared means of *C. annuum* heights at three and five weeks after planting with respect to environment of origin. Shaded bars indicate 95% confidence intervals and means sharing a letter are not significantly different using a Tukey test. Howlett Greenhouse, The Ohio State University, 2020.



a) Height at 5 weeks after planting

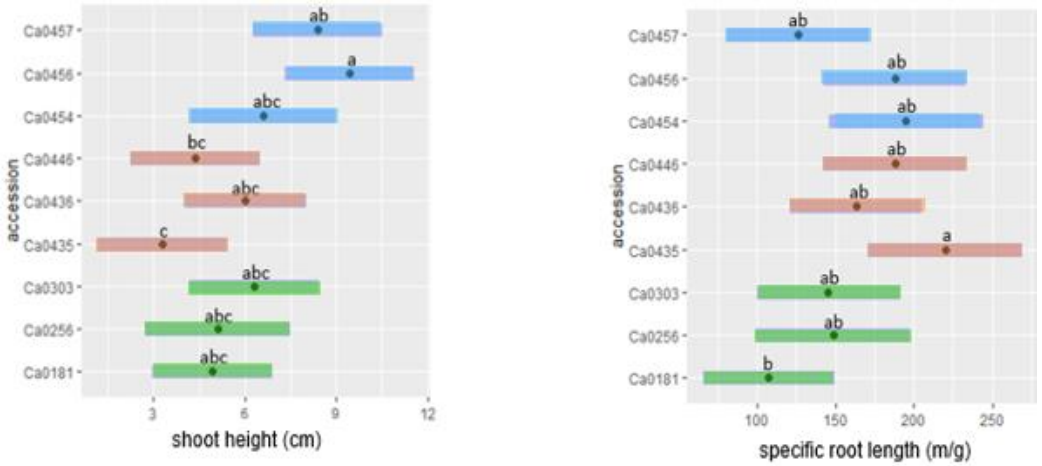
b) Shoot dry weight+

c) Total root length

d) Root dry weight

e) Average root diameter

Figure 4: Least squared means of *C. annuum* heights, shoot dry weights, total root lengths, root dry weights, and average root diameters with respect to treatment. Shaded bars indicate 95% confidence intervals and means sharing a letter are not significantly different using a Tukey test. ┼ indicates data transformed by square root. Howlett Greenhouse, The Ohio State University, 2020.



a) Height at 5 weeks after planting

b) Specific root length

Figure 5: Least squared means of *C. annuum* heights at five weeks after planting and specific root lengths with respect to accession. Shaded bars indicate 95% confidence intervals and means sharing a letter are not significantly different using a Tukey test. Blue bars indicate accessions from environments of moderate precipitation; pink bars indicate accessions from environments of high precipitation; green bars indicate accessions from environments of low precipitation. Howlett Greenhouse, The Ohio State University, 2020.

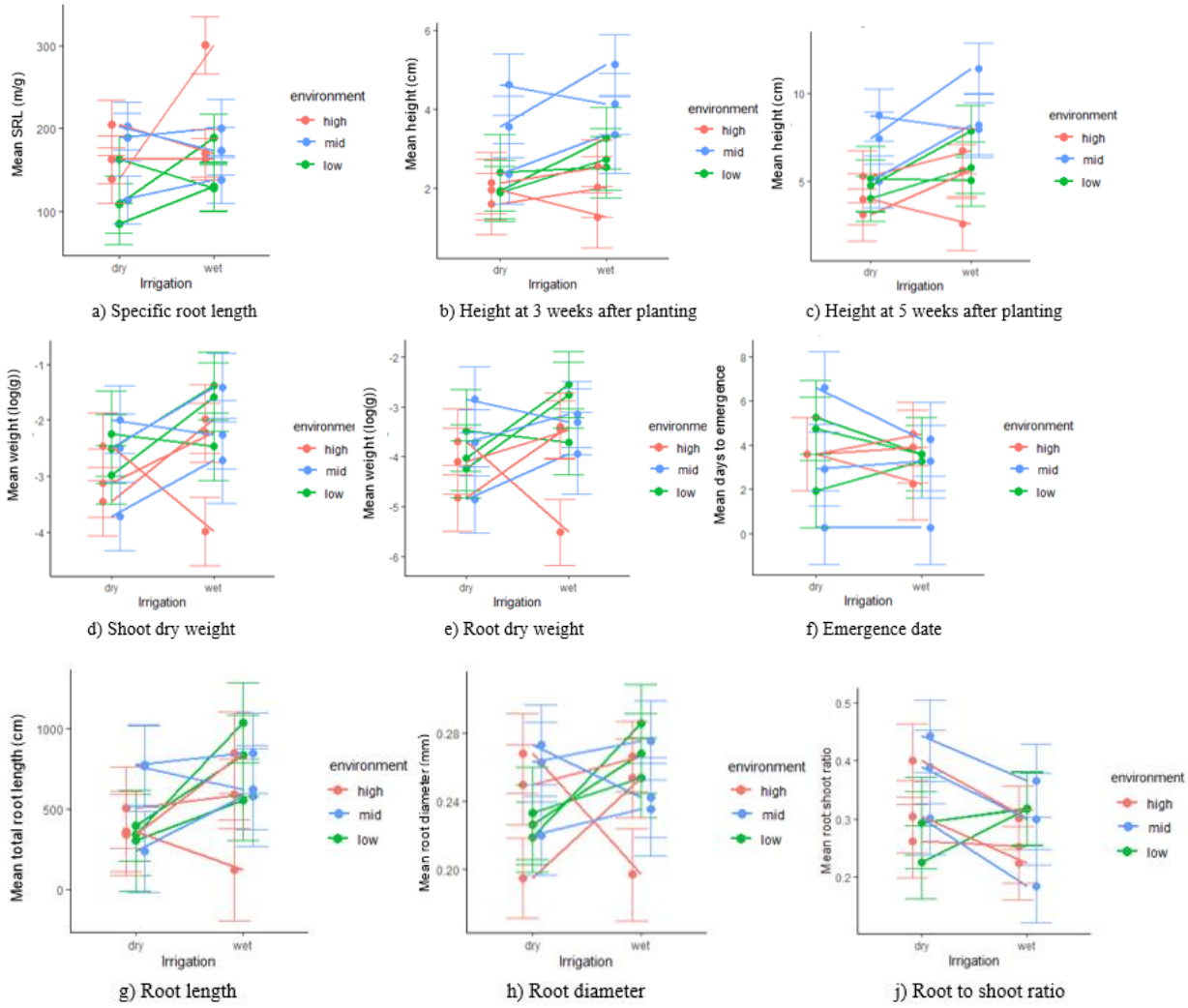


Figure 6: Interactions of treatment and accession with regard to specific root length, height at three and five weeks after planting, shoot and root dry weight, emergence date, root length and diameter, and root to shoot ratio in *C. annuum.* Points indicate least squared means; bars indicate standard errors. Howlett Greenhouse, The Ohio State University, 2020.